



RESEARCH ARTICLE

Physiological and biochemical responses of Jew's mallow (*Corchorus olitorius* L.) to foliar spray of nanosized ZnO

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Abstract

Despite the positive impact of nanomaterials on agriculture and crop productivity, this effect is not always positive. A pot experiment was undertaken to spot the effect of nanosized ZnO on the physiological and biochemical attributes of Jew's mallow (*Corchorus olitorius* L.) by foliar spray applied at three concentrations (25, 50, and 100 mg L⁻¹) in addition to the control. All concentrations, especially 100 mg L⁻¹ of ZnO significantly increased ($p \leq 0.05$) plant growth parameters, compared to the control. Protein, carbohydrates and fibers were increased after the application of ZnO NPs by 47, 77 and 94% respectively while fat was not changed. Likewise, significant variations in element contents (N, P, K, Zn and Fe) occurred following the nanosized ZnO application. Moreover, nanosized ZnO induced the activity of catalase and ascorbate peroxidase enzymes and the highest levels were (0.82 and 3.14 U g⁻¹ FW min⁻¹ respectively) recorded at 100 mg L⁻¹ of ZnO whereas, causing inhibition in H₂O₂ and lipid peroxidation content by (9.3 and 31.6 % respectively). Hence, nanosized ZnO can improve plant growth and the nutritive value of Jew's mallow and can induce tolerance of the plant against oxidative stress.

Keywords

ascorbate peroxidase; catalase; element content; lipid peroxidation; nutritive value; ZnO NPs

Introduction

Humans rely on plants as food sources, and the quality of their food influences their health. Recently, nanotechnology has had immense applications in food security and healthy nutrition. It is being used in agriculture for many goals and under different conditions (1). Utilization of nanomaterials in agriculture primarily aims to decrease toxic chemicals, restoring nutrient losses and boosting the yield of the crop (2). Nanotechnology can improve the nutritional values of crops by using some engineered nanoparticles as a fertilizer (3). Nanosized zinc oxide is one among the most widely used worldwide which has positive and negative effects on the ecosystem (4).

In the field of agriculture, nanozinc oxide has numerous applications as it is used as a fertilizer in crop production that helps the soil to restore the lost nutrients, and is applied in crops modified genetically and nanofoods as part of diets required for some patients (5). Sabir *et al.*, reported the ability of ZnO NPs in enhancing the growth and yield of crops (6). Nanosized ZnO is effective in promoting seed germination and plant growth as well as prevention of disease (7). Nandhini *et al.*, confirmed ZnO NPs'

positive effect on the germination and growth of pearl millet (8). Additionally, Singh *et al.*, (9) and Rizwan *et al.*, (10) demonstrated their impacts on the quantitative, nutritional, and physiological parameters of wheat and maize. Zinc is necessary for optimum plant growth and agricultural yield in low concentrations, while higher concentrations of ZnO NPs can stunt plant growth by affecting seed germination, root development, and seedling biomass (11). However, its insufficiency could obstruct the growth and development of plants; moreover, its deficiency could have harmful consequences on humans, thus it is crucial to increase crops' uptake of Zn (9). The use of Zn as foliar fertilizer has been approved to be an efficient method to overcome the problem of Zn binding in soil (12) and ensure the nutrients supply to different plant organs required for critical growth stages (13). Concerning the nutrients supply to plants, Dimkpa *et al.*, proved that foliar application is more effective than conventional fertilization and also gives smaller quantities in a controlled manner (14).

Jew's mallow (*Corchorus olitorius* L.) is one of the most important leafy vegetables in tropical regions including Egypt. In Egypt, especially Upper Egypt, it is utilized to bring daily revenue for small holders and low-income people. This plant is significant in the human diets of Asia and Africa because it can provide protein, vitamins, minerals, and energy (15). Jew's mallow leaves contain vitamins, minerals (Ca, Mg and Fe), dietary fiber, protein, folic acid and ascorbic acid (15). Moreover, Jew's mallow can prevent disease because of its antioxidant, antihypertensive, anti-diabetic, and anti-ulcer properties (16). However, research activities aimed at enhancing growth, yield and nutritional qualities of this crop received less attention so far. The cultivation of Jew's mallow in marginal areas without adding any fertilizers or small amounts of organic and/or inorganic fertilizers to the plant, foliar application of fertilizers, caused a great reduction in crop yield and quality (17). Using nanomaterials in Jew's mallow cultivation has not received adequate attention. The effect of nanosized ZnO on the growth, nutrient uptake and antioxidant activity need proper understanding and documentation. Therefore, this study was undertaken to assess the physiological and biochemical responses of Jew's mallow to the nanosized ZnO. The potential to obtain a more sustainable nano technology for the crop was also investigated.

Materials and Methods

Preparation of ZnO NPs

ZnO NPs stock suspension (1000 mg L⁻¹) was prepared by dispersing a known weight of ZnO NPs in deionized water (w/v). Three concentrations (25, 50, and 100 mg L⁻¹) of ZnO NPs were prepared from the stock solution and chosen according to previous study carried out by Munir T, *et al* (18). First, white powder was obtained by utilizing zinc acetate and NaOH as precursors in the sol-gel method to give ZnO nanoparticles. The characterizations of ZnO have been investigated by X-ray diffraction (XRD) and transmission electron microscope (TEM).

Pot experiment

A pot experiment was carried out in early April 2022 by using clay soil with pH 8, field capacity 42%, available P 0.01%, and total N 0.08%. The seeds of *C. olitorius* (cv. Balady) were selected from the Agricultural Research Center of Egypt. The pots were arranged in a completely randomized block design with four treatments including control and three replicates for each treatment.

ZnO NPs foliar spray was applied 3 times every 10 days during the vegetative growth. The spraying solution should be cover the plant foliage completely. The foliar spray method was selected because essential elements were absorbed worthily through leaves and to avoid soil obstructions. After 45 days, plants were collected for further analysis.

Growth characteristics

Growth parameters of vegetative stage were measured such as plant height (cm), leaves number, fresh and dry weights of the shoot (g), and leaf area (cm²).

Measurement of basic nutrients

The content of total protein was estimated using Bradford method using 10 ml of a 25 mM borate buffer solution (pH 8.5) for protein extraction and Coomassie brilliant blue (G250) as a protein reagent (19). The absorbance was measured and Bovine Serum Albumin (BSA) was used as a standard for the calculation of protein concentrations in terms of mg g⁻¹DW.

Total carbohydrates content was determined by the method according to Dubois M, *et al.*, using phenol sulphuric acid at 490 nm absorbance (20). A glucose standard curve was used for calculating the amount of total soluble carbohydrates as mg g⁻¹DW.

Fats were determined by the soxhlet fat extraction method using petroleum ether at 60-80° C (21). Total dietary fiber determined according to Prosky L. (22). The samples were enzymatically digested with α-amylase and then with protease and amyloglucosidase. After digestion, the total fiber content was precipitated by adding 95% ethanol. Then the solution was filtered and fiber was collected, dried, and weighed.

Estimation of elements content

The concentration of elements (N, P, K, Zn and Fe) in Jew's mallow was determined according to Motsara and Roy (23). Samples were cleaned with fresh and distilled water and then dried in an oven at 65°c for 24 h and digested in 10 ml acids mixture (1HNO₃ + 3 HCl). The elements were measured by flame photometer Shimadzu Model AA 640 F (Japan).

Determination of antioxidant enzymes, H₂O₂ and lipid peroxidation

The leaves of Jew's mallow were powdered in n 0.05 M phosphate buffer (pH 7.0) with 1 mM EDTA, and the mixture was centrifuged at 10,000 rpm for 10 minutes. As an enzyme source, the supernatant was completed to a known volume. Catalase activity (CAT) was measured using a method described by (24). Ascorbate peroxidase

(APOX) activity was prepared and assayed according to the method used by Nakano and Asada (25).

H₂O₂ content was evaluated by the method of Gay and Gebicki (26). In liquid nitrogen, fresh weight of leaves was grounded and then homogenised in 4 mL of a solution containing 100 mM potassium phosphate (pH 6.8). A final concentration of 25 mM H₂SO₄, 100–150 mM xylene orange, and 100–250 mM ferrous ammonium sulphate in a volume of 2 mL was obtained after diluting the combination. After 30 minutes of dark incubation, the absorbance at 560 nm was measured using XO/Fe²⁺ as a blank.

Lipid peroxidation content was estimated by measuring the MDA concentration using thiobarbituric acid depending on the method of (27). Using a spectrophotometer, absorbance was measured at 450, 532, and 600 nm, respectively, and its concentration was determined using the following formula: $MDA = 6.45(A_{532} - A_{600}) - 0.56 A_{450}$

Statistical analysis

One-way ANOVA was used for analyzing data depending on means and standard deviation (SD) values. The p-value ≤ 0.05 was considered statistically significant using a MSTAT-C statistical analysis package and followed by post hoc test using Duncan Multiple Range (DMR) test for comparisons between means.

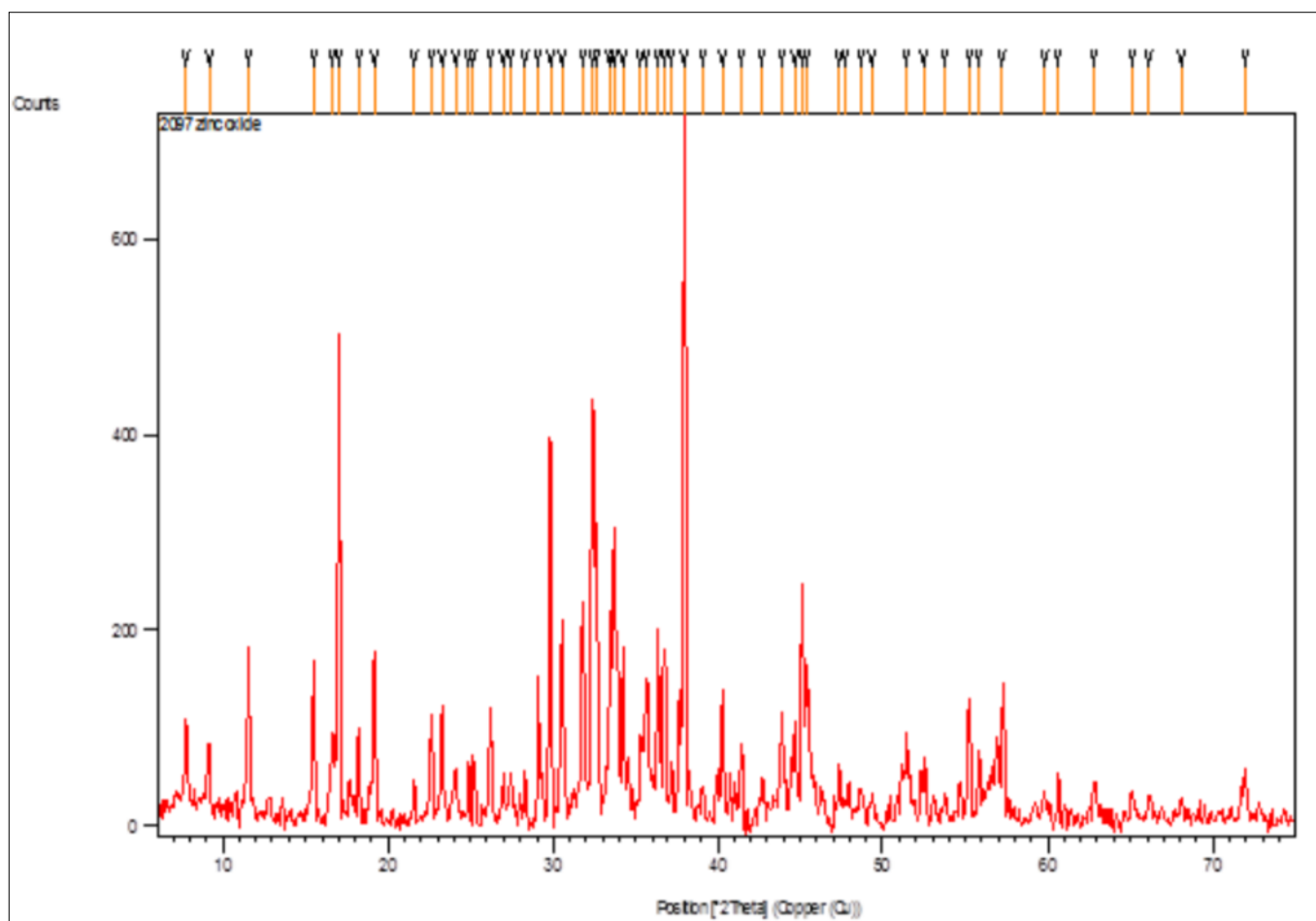
Results and discussion

Both XRD pattern and TEM image of ZnO NPs have been shown in Fig. 1. The XRD pattern confirms the formation of

NPs and these particles are observed to be highly crystalline. Peaks at 2θ of 19.16°, 30.53°, 32.39°, 33.68°, 37.98° were referred to d-spacing (Å) 4.628, 2.925, 2.761, 2.658, 2.366. The XRD results confirmed the formation of ZnO nanoparticles (Fig.1 A). The TEM image demonstrates different shape and size of NPs. This TEM image also contains different kinds of particles such as bunch of irregular particles, smaller particles, round shape particles. Complex nanostructure is formed due to different size and shape of NPs. This complex nanostructure image is distributed over the whole scanned area. The average crystalline size of ZnO NPs is 9.89 nm (Fig1 b).

Growth characteristics

Growth parameters of the vegetative growth included plant height, leaves numbers, leaf surface area, fresh and dry weights of shoot are shown in (Table 1). These parameters are linearly increased with increasing concentrations of ZnO NPs foliar spray. The highest values of growth parameters were recorded at 100 mg L⁻¹ZnO NPs (the highest concentration) foliar treatment, whereas the lowest values of these parameters were recorded in control. Changes of plant growth parameters observed with different treatments were statistically significant ($p \leq 0.05$). Maximum values of plant height (20.01 cm), shoot fresh wt. (14.98 g), shoot dry wt. (9.11 g), leaves number (18.33) and leaf area (19.90 cm²) were recorded at 100 mg L⁻¹ZnO NPs which significantly higher than other treatments and control (Table 1). Under the effect of nanosized ZnO foliar spray, all growth characteristics showed a statistically significant



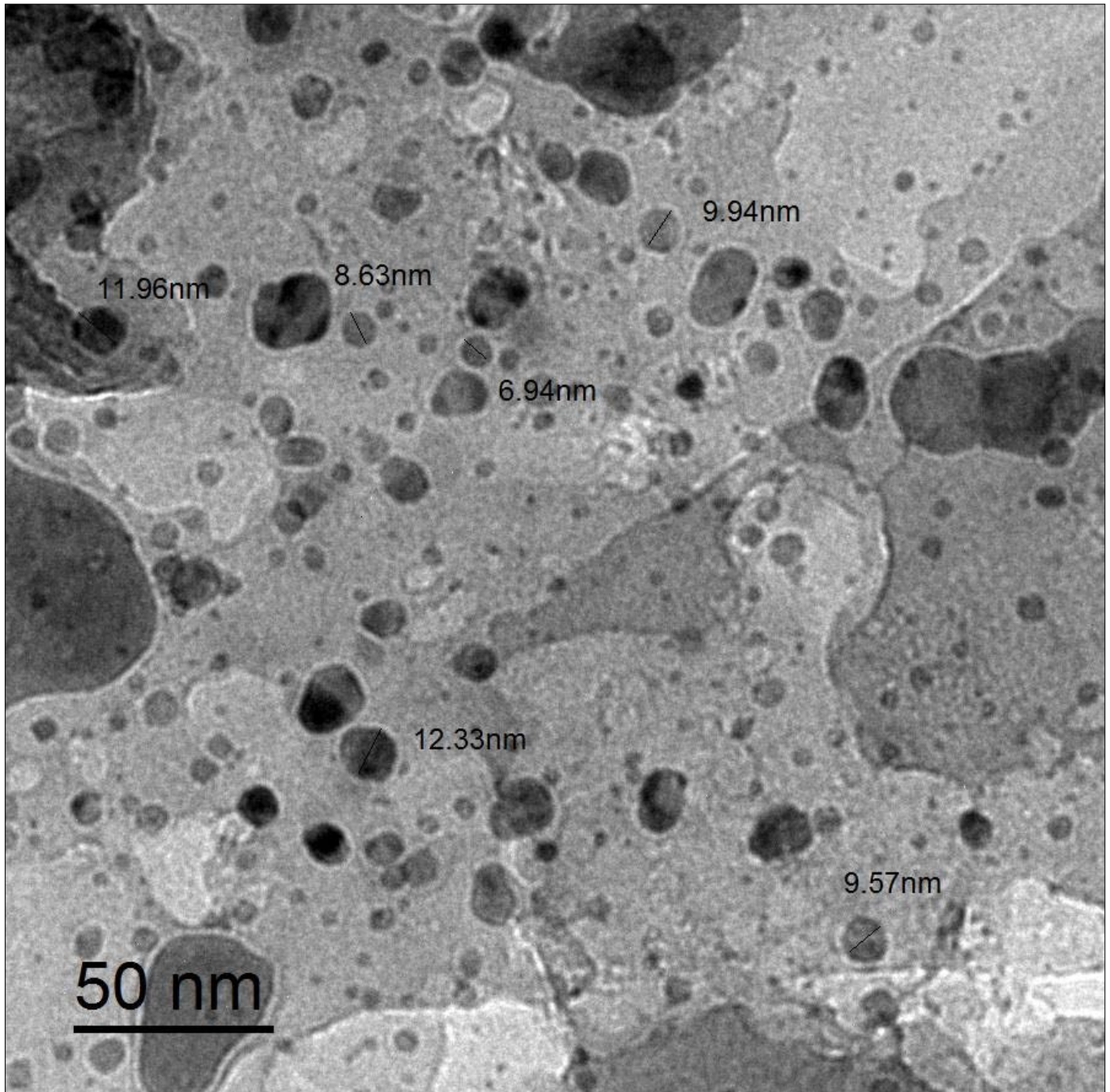


Fig. 1. XRD pattern (A) and TEM image (B) of ZnO NPs.

Table 1. Growth characteristics of *C. olitorius* under effect of different concentrations of ZnO NPs.

ZnO NPs conc. (mg L ⁻¹)	Plant height (cm)	Leaves no.	Shoot fresh wt. (g)	Shoot dry wt. (g)	Leaf surface area (cm ²)
0	14.60 ± 0.341 ^d	8.33 ± 0.577 ^d	11.48 ± 0.57 ^d	6.83 ± 0.331 ^c	16.37 ± 0.330 ^c
25	16.08 ± 0.310 ^c	11 ± 1 ^c	12.67 ± 0.406 ^c	7.09 ± 0.987 ^{bc}	16.75 ± 0.232 ^c
50	19.15 ± 0.658 ^b	14.66 ± 1.154 ^b	13.91 ± 0.353 ^b	8.04 ± 0.066 ^b	17.96 ± 0.461 ^b
100	20.01 ± 0.405 ^a	18.33 ± 1.527 ^a	14.98 ± 0.378 ^a	9.11 ± 0.296 ^a	19.90 ± 0.555 ^a
Significance	*	*	*	*	*

Mean ± standard error based on ANOVA analysis. Means in the same row followed by different letters in each column are significantly at the 5% probability level (p value at 0.05) according to Duncan Multiple Range Test (DMRT).

increase especially at concentration 100 mg L⁻¹ which had the maximum impact on plant growth, probably because this concentration is a suitable level of nanosized Zn required for seedling growth of Jew's mallow. This is related to the importance of zinc in cell elongation, membrane

function, and protein synthesis might be leading to the enhancement of plant growth than the control. Previous studies proved that nanosized ZnO foliar spray increased shoot biomass, plant growth, and protein content (28,29). Results of this study showed that ZnO NPs have a positive

role on the growth of Jew's mallow. This might be attributed to the role of Zn as a cofactor for many enzymes, ultra-small size and easy solubility. This is in addition to its diffusible nature with high capacity to leaf surface penetration and release of Zn ions across the cuticle. Occasionally, foliar spray method has proven to be the effective method as it can release fertilizer gradually and slowly (14).

Basic nutrients

The effect of foliar nanosized ZnO doses produced increases in protein, carbohydrate and fiber contents. There is a high positive correlation between these nutrients content and concentrations of ZnO NPs. These nutrients increased with increasing concentration of ZnO NPs. 100 mg L⁻¹ZnO NPs recorded the maximum increase of protein, carbohydrate and fiber and the percentages were 47, 77 and 94 respectively compared to the control (Fig. 2). Fat content showed no differences between the concentrations, where recorded 2.87, 2.88 and 2.90 mg g⁻¹ DW at 25, 50 and 100 mg L⁻¹ZnO NPs, respectively. Except for fat which was not affected by ZnO NPs foliar spray compared to control (Fig. 2. D), nanosized ZnO had a significant impact on the other basic nutrients, protein, carbohydrate, and fiber contents (Fig. 2. A, B, C). Data of the present study showed that ZnO NPs foliar spray significantly increased protein, carbohydrate, and fiber contents in Jew's mallow compared to control. Different treatments of nanosized ZnO increases

the carbohydrate content in Jew's mallow, and the 100 mg L⁻¹ZnO NPs produced the highest carbohydrate content. Zinc is involved in starch formation because of the enzymes of carbohydrate metabolism depend on Zn (30). Moreover, higher protein contents have been recorded under effect of different concentrations of nanosized ZnO, this might be attributed to the critical role of Zn in protein synthesis (31). These results were consistent with results provided by Kisan B *et al.*, on spinach (32). Generally, the efficiency of photosynthesis is significantly increased by metal nanoparticles which may be related to an increase in the concentration of carbohydrates. Likewise, zinc can control auxin, plant growth hormone, and help in the metabolism of carbohydrates and proteins (33). Lambot reported that the nutritional content of crops is based on its protein and carbohydrate content and the protein content is the greatest advantage for human consumption (34). Foliar application of nanosized ZnO makes Zn more available during the vegetative growth which is effective to the physiological functions that could improve the nutritional quality (10).

Element content

In this study, the effect of ZnO NPs on the nutritional status of Jew's mallow during the vegetative stage was evaluated. Foliar spray of ZnO NPs caused significant variations in the nutrient contents, N, P, K, Fe and Zn (Table 2). Results showed a significant increase in the accumulation of

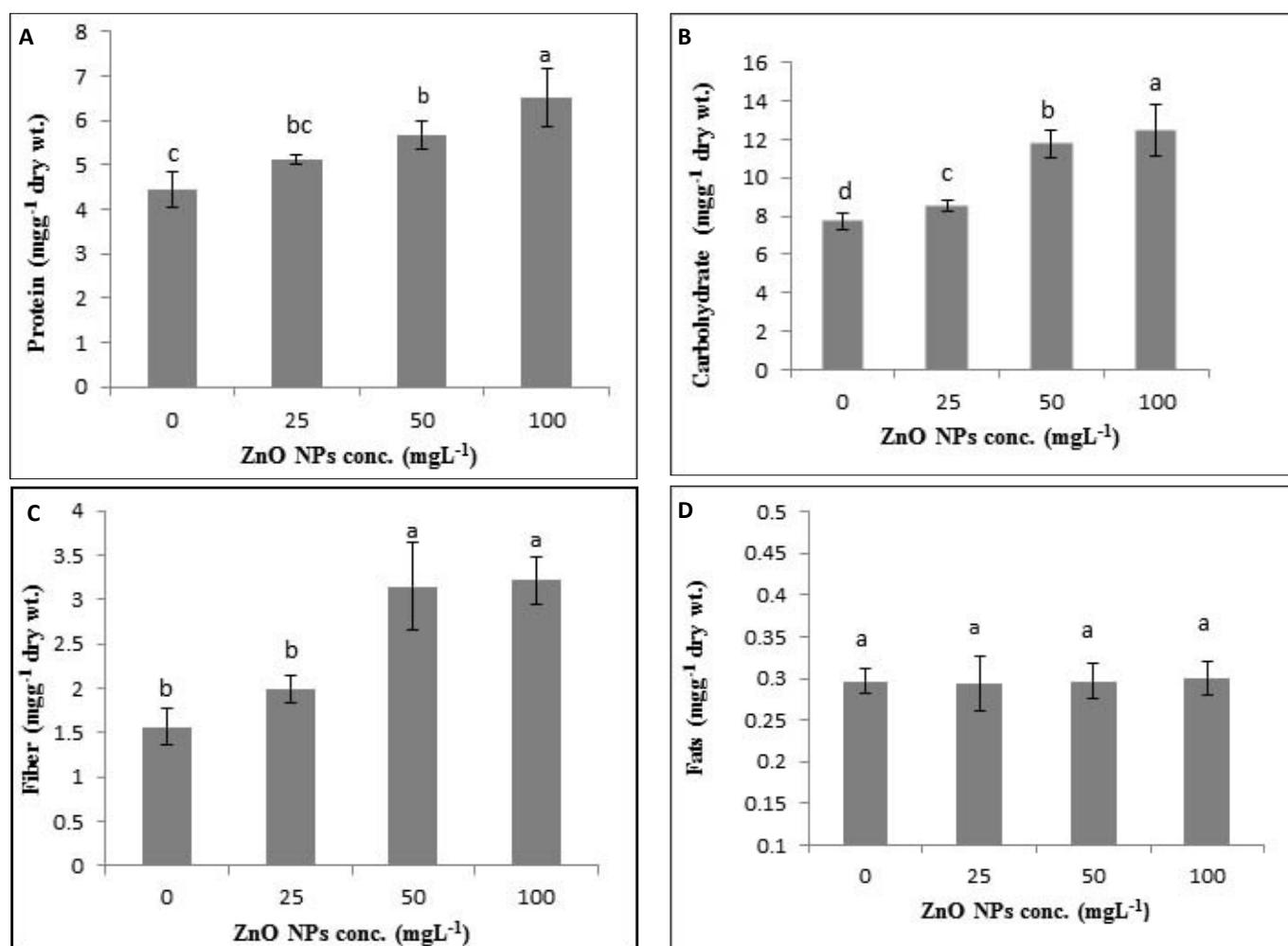


Fig. 2. Basic nutrients (mg g⁻¹ DW) under effect of different concentrations of nanosized ZnO, protein (A), carbohydrate (B), fiber (C) and fats (D). Error bars expressed as different letters are significantly different among treatments at P ≤ 0.05 according to Duncan Multiple Range Test (DMRT).

Table 2. Macro and micro nutrients (mg g⁻¹ DW) of *C. olitorius* under effect of different concentrations of ZnO NPs.

ZnO NPs conc. (mg L ⁻¹)	N	P	K	Zn	Fe
0	45.78 ± 0.91 ^d	103.06 ± 1.22 ^a	437.9 ± 0.40 ^d	11.69 ± 0.32 ^c	2.77 ± 2.08 ^a
25	48.47 ± 0.60 ^c	98.13 ± 0.46 ^b	439.02 ± 0.24 ^c	12.80 ± 0.42 ^c	2.81 ± 2.8 ^a
50	52.88 ± 0.96 ^b	94.41 ± 0.71 ^c	441.38 ± 0.17 ^b	17.33 ± 0.38 ^b	2.70 ± 1.96 ^a
100	56.57 ± 0.73 ^a	90.62 ± 0.38 ^d	442.68 ± 0.28 ^a	19.49 ± 0.37 ^a	2.83 ± 1.57 ^a
Significance	*	*	*	*	*

Mean ± standard error based on ANOVA analysis. Means in the same raw followed by different letters in each column are significantly at the 5% probability level (p value at 0.05) according to Duncan Multiple Range Test (DMRT).

nitrogen content particularly with the highest level of ZnO NPs. The highest value of N was 56.57 mg g⁻¹ recorded at 100 mg L⁻¹ ZnO NPs compared to control. Zn has favorable effects on the bioavailability of nutrients in addition to improving the efficiency of the root of cation exchange so as to lead towards an increase in the absorption of nutrients, especially N which is responsible for high level of protein (35). On the other hand, a significant decrease in phosphorus content was found under effect of ZnO NPs. The highest value of phosphorus was 103.06 mg g⁻¹ observed at control, more than other treatments (25, 50 and 100 mg L⁻¹) which recorded 98.13, 94.41 and 90.62 mg g⁻¹ DW respectively. Zn decreased the uptake of phosphorus that may be the antagonistic relation between zinc and phosphorus (36). Additionally, this agrees with the hypothesis of zinc application interrupts the absorption and translocation of some nutrients namely, phosphorus (37). The determined variations of iron content was not significant, there was no difference in iron content between different concentrations of ZnO NPs and control (Table 2). Even though zinc has an antagonistic effect on some elements of two capacity cations like iron (38), the content of iron was not significantly changed with increase in the content of zinc. Iron is involved in the activation of many metabolic, physiological and biochemical pathways in plants, and it acts as a component of many enzymes' prosthetic groups (39).

Similarly, both contents of potassium and zinc increase with increasing the concentration of ZnO NPs. The highest values of potassium and zinc were 442.68 and 19.49 mg g⁻¹ respectively at 100 mg L⁻¹ compared to control (Table 2). This study showed improvement in potassium and zinc contents in Jew's mallow sprayed with ZnO NPs. Although, potassium is not a constituent of any plant structures but it plays a part in many important regulatory roles in the plant such as osmoregulation process,

regulation of plant stomata, translocation of sugars, energy status of the plant, regulation of enzyme activities (40). Zinc is required for germination and plays an important role in membrane integrity as well as the synthesis of proteins and some phytohormones (40). Hence, deficiency of zinc can lead to negative effects on all actors of the whole chain, notably humans. This results in the impetus on the importance to improve the uptake of zinc by crops and subsequently humans. The present findings are also consistent with the studies done earlier which show that about three times increase in zinc uptake by corn (41) and by green peas (42), treated with ZnO NPs.

Contents of antioxidative enzymes, H₂O₂ and lipid peroxidation

Results presented in Table (3) show the effect of nanosized ZnO in different doses on oxidation system of Jew's mallow and on the antioxidant enzymes. The oxidative stress indicated by H₂O₂ concentration and lipid peroxidation content in the plant, whereas the antioxidative system determined by CAT and APOX activity. The application of ZnO NPs induced the activity of CAT and APOX in the treated plants, the highest activity (0.82 and 3.14 U g⁻¹ FW min⁻¹ respectively) recorded at 100 then at 50 and 25 mg L⁻¹ compared to control which record 0.064 and 2.51 U g⁻¹ FW min⁻¹ respectively (Table 3). In this study, a significant increase (p ≤ 0.05) in antioxidant enzymes activity after nanosized ZnO application could be the sign of buildup of a protective means to lessen the oxidative stress. NPs improve antioxidant activity in tissues and result in increased production of secondary metabolites (43). These phytochemicals are responsible of the neutralization of toxic free radicals and prevention of excessive oxidation reactions (44). It has been demonstrated that the use of ZnO NPs increases the expression of important antioxidant stress-responsive

Table 3. Effect of different concentrations of ZnO NPs on antioxidative enzymes, H₂O₂ concentration and lipid peroxidation.

ZnO NPs conc. (mg L ⁻¹)	CAT enzyme (U g ⁻¹ FW min ⁻¹)	APOX enzyme (U g ⁻¹ FW min ⁻¹)	H ₂ O ₂ (µg g ⁻¹ DW)	Lipid peroxidation (µg g ⁻¹ DW)
0	0.064 ± 0.001 ^a	2.51 ± 0.017 ^a	1.49 ± 0.004 ^c	12.34 ± 0.24 ^d
25	0.066 ± 0.000 ^{ab}	2.80 ± 0.002 ^b	1.46 ± 0.005 ^{bc}	11.15 ± 0.31 ^c
50	0.073 ± 0.002 ^c	2.89 ± 0.025 ^c	1.42 ± 0.004 ^b	9.26 ± 0.06 ^b
100	0.82 ± 0.0018 ^d	3.14 ± 0.001 ^d	1.35 ± 0.012 ^a	8.43 ± 0.21 ^a
Significance	*	*	*	*

Mean ± standard error based on ANOVA analysis. Means in the same raw followed by different letters in each column are significantly at the 5% probability level (p value at 0.05) according to Duncan Multiple Range Test (DMRT).

enzymes in *G. hirsutum* (45) and *O. sativa* (46). CAT and APOX are enzymatic scavengers of activated oxygen so, they are important in defense system of plants. Both enzymes can convert the hydrogen peroxide to water and oxygen, so involved in the detoxification of H_2O_2 . H_2O_2 was removed and lipid peroxidation was inhibited as a result of ZnO NPs' stimulation of the synthesis of antioxidative enzymes (47). It was noted that H_2O_2 concentration after ZnO NPs application was lower than non treated plants. The lowest value of H_2O_2 concentration at 100 mg L^{-1} which decreased by 9.3 %. Non-treated plants (control) showed the highest value of H_2O_2 ($1.49\mu\text{g g}^{-1}\text{ DW}$). A similar trend was observed in lipid peroxidation content (MDA), where the content decreased as ZnO NP concentrations increased. The highest value of MDA content was $12.34\mu\text{g g}^{-1}\text{ DW}$, observed at control. MDA content in the treated plants was lower than the non treated plants. ZnO NPs reduced MDA content by 31.6 %. Lipid peroxidation is the process whereby free radicals steal electrons from the lipids in cell membranes, this causes a free radical chain reaction mechanism that demonstrates the magnitude of the oxidative stress, which damages cells and produces MDA (48). The reduction in its content in Jew's mallow following ZnO NPs application might be due to ZnO NPs' support for plant production of antioxidant enzymes that reduce ROS before peroxidation. These results are in line with (49) who reported that ZnO NPs treatment reduce lipid peroxidation, and induced antioxidant enzymes in *L. leucocephala*. Generally, improvement of physiological and biochemical attributes by nanomaterials application considered as a unique technique. The nanosized ZnO used in fertilization of many crop plants. This study spots more light on the safe use of ZnO as a nanofertilizer on the growth, nutrient content and antioxidant activity of Jew's mallow. Besides the determination of ZnO NPs concentrations that are possibly improving these attributes, which bring insight at the choice of concentration to apply. It is well known that micronutrient fertilizers like nanoparticles are used to prevent fertilizer-related pollution since they are effective at supplying the necessary nutrients gradually and under controlled conditions (14). The effects of Zn ions and ZnO NPs rely on the concentration at which they are applied as well as the biological characteristics of plant species, such as the permeability of seed coat to NPs and their internalization in root tissues (50).

Conclusion

Most rural communities in low-income countries and other parts of Africa rely on vegetables as a source of protein, iron, and β -carotene; therefore, Jew's mallow could play a major role in supplying rural communities with cheap and nutritious protein. It can substantiate these nutrients in rich quantities in the human diet that will help the poor population to fight against hunger and malnutrition. This study provides the critical concentration of ZnO nanofertilizer for better growth of Jew's mallow. The role of ZnO NPs may improve the physiological and biochemical properties of the plant as well as the tolerance of oxidative stress. ZnO nanosized foliar application improved the

contents of protein, carbohydrate and fibers without affecting fat which made Jew's mallow is more nutritive and recommended to the vegetarian diet. However, further analyses are needed to explore the physiological mechanisms of ZnO NPs and their interference with the metabolic pathways in plants specifically, stress response system.

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Compliance with ethical standards

Conflict of interest: Author does not have any conflict of interests to declare.

Ethical issues: None.

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